

APPLICANTS: Rush *et al.*
U.S.S.N.: 10/777,893

Listing of Claims

1. (currently amended) A method for isolating a post-translationally modified peptide from a complex mixture of peptides, said method comprising the steps of:
 - (a) obtaining a proteinaceous preparation from an organism, wherein said proteinaceous preparation comprises post-translationally modified peptides from two or more different proteins;
 - (b) contacting said proteinaceous preparation with at least one immobilized post-translational modification-specific antibody; and
 - (c) isolating at least one post-translationally modified peptide specifically bound by said immobilized modification-specific antibody in step (b).
2. (original) The method of claim 1, further comprising the step of (d) characterizing said modified peptide isolated in step (c) by mass spectrometry (MS), tandem mass spectrometry (MS-MS), and/or MS³ analysis.
3. (currently amended) The method of claim 2, wherein said mass spectrometry comprises matrix-assisted laser desorption time-of-flight (MALDI-TOF) MS, wherein said tandem mass spectrometry comprises liquid chromatography (LC)-MS/MS, and wherein said MS³ analysis comprises LC-MS³.
4. (original) The method of claims 2 or 3, further comprising the step of (e) utilizing a search program to substantially match the spectra obtained for said modified peptide during the characterization of step (d) with the spectra for a known peptide sequence, thereby identifying the parent protein(s) of said modified peptide.
5. (original) The method of claim 1, wherein said proteinaceous preparation comprises a digested biological sample selected from the group consisting of a digested crude cell extract, a digested tissue sample, a digested serum sample, a digested urine sample, a digested synovial fluid sample, and a digested spinal fluid sample.
6. (original) The method of claim 5, wherein said digested preparation is obtained using at least one proteolytic enzyme or chemical cleavage.
7. (original) The method of claim 6, wherein said proteolytic enzyme is immobilized.

APPLICANTS: Rush *et al*
U.S.S.N.: 10/777,893

8. (original) The method of claim 6, wherein said proteolytic enzyme is soluble, and wherein said digested preparation is treated with a proteolysis inhibitor prior to said contacting step (b).
9. (original) The method of claim 1, wherein step (a) further comprises pre-purifying said proteinaceous preparation by immobilized metal affinity chromatography (IMAC).
10. (original) The method of claim 1, wherein said immobilized antibody of step (b) is covalently-linked to a chromatography resin or noncovalently-linked to protein-A- or protein-G-agarose.
11. (original) The method of claim 10, wherein said resin is contained within a column or micropipette tip.
12. (original) The method of claim 2, wherein said immobilized antibody of step (b) is immobilized in chromatography resin within a column, said column being coupled to a mass spectrometer for said characterization of step (d).
13. (original) The method of claim 1, wherein said modification comprises phosphorylation.
14. (original) The method of claim 1, wherein said modified peptide(s) comprise(s) a phosphopeptide.
15. (currently amended) The method of claim 1, wherein said modification-specific antibody comprises a motif-specific, context-independent antibody that specifically binds ~~recognizes~~ a motif comprising at least one phosphorylated amino acid.
16. (original) The method of claim 15, wherein said motif consists of a single phosphorylated amino acid.
17. (currently amended) The method of claim 15, wherein said motif consists of ~~comprises~~ all or part of a kinase consensus substrate motif or a protein-protein binding motif.
18. (currently amended) The method of claim 17, wherein said kinase consensus substrate motif is selected from the group consisting of mitogen-activated protein kinase (MAPK) consensus substrate motifs, cyclin-dependent kinase (CDK) consensus substrate motifs, protein kinase A (PKA) consensus substrate motifs, AKT consensus substrate motifs, protein kinase C (PKC) consensus substrate motifs, phosphothreonine-X-arginine, and ATM (ataxia telangiectasia mutated) consensus

APPLICANTS: Rush *et al.*
U.S.S.N.: 10/777,893

substrate motifs, and wherein said protein-protein binding is a 14-3-3 binding motif or a 3-phosphoinositide-dependent kinase 1(PDK1) docking motif.

19. (original) The method of claim 1, wherein said modification-specific antibody is a monoclonal antibody or a polyclonal antibody.
20. (original) The method of claim 1, wherein said modified peptide isolated in step (c) corresponds to a known marker of disease.
21. (original) The method of claim 4, wherein said modified peptide characterized in step (d) comprises an unknown modification site of said parent protein.
22. (original) The method of claims 2 or 3, further comprising the step of (e) comparing the modification state of said modified peptide characterized in step (d) with the modification state of a corresponding peptide in a reference sample, thereby to compare protein activation in said proteinaceous preparation with protein activation in said reference sample.
23. (original) The method of claim 22, wherein said proteinaceous preparation corresponds to a diseased organism and said reference sample corresponds to a normal organism, whereby comparison of protein activation provides information on activation changes resulting from said disease.
24. (original) The method of claim 22, wherein said proteinaceous preparation is obtained from a tissue biopsy cell or a clinical fluid sample and said reference sample corresponds to a diseased organism, whereby the comparison of protein activation provides information useful for diagnosis of said disease.
25. (original) The method of claim 22, wherein said protein preparation corresponds with an organism or preparation treated with at least one test compound and said reference sample corresponds with an untreated organism or preparation, whereby the comparison of protein activation provides information on activation changes resulting from treatment with said test compound.
26. (original) The method of claim 23, wherein the comparison of protein activation identifies the modified peptide characterized in step (d) as corresponding to a parent protein not previously reported as so modified in said disease.
27. (original) The method of claim 24 or 25, wherein said disease is cancer.

APPLICANTS: Rush *et al*
U.S.S.N.: 10/777,893

28. (original) The method of claim 25, wherein said test compound comprises a cancer therapeutic.
29. (original) The method of claim 25, wherein said test compound comprises a kinase inhibitor.
30. (currently amended) A method for isolating a phosphopeptide from a complex mixture of peptides, said method comprising the steps of:
- (a) obtaining a proteinaceous preparation from an organism, wherein said proteinaceous preparation comprises phosphopeptides from two or more different proteins;
 - (b) contacting said proteinaceous preparation with at least one immobilized motif-specific, context-independent antibody that binds a motif comprising at least one phosphorylated amino acid;
 - (c) isolating at least one phosphopeptide specifically bound by said immobilized antibody in step (b); and
 - (d) characterizing said ~~modified~~ phosphopeptide isolated in step (c) by mass spectrometry (MS), tandem mass spectrometry (MS-MS), and/or MS³ analysis.
31. (currently amended) The method of claim 30, further comprising the step of (e) utilizing a search program to substantially match the mass spectra obtained for said ~~modified~~ phosphopeptide during the characterization of step (d) with the mass spectra for a peptide of one or more known protein(s), thereby identifying the parent protein(s) of said ~~modified~~ phosphopeptide.
32. (currently amended) The method of claim 32, wherein said mass spectrometry comprises matrix-assisted laser desorption time-of-flight (MALDI-TOF) MS, wherein said tandem mass spectrometry comprises liquid chromatography (LC)-MS/MS, and wherein said MS³ analysis comprises LC-MS³.
33. (original) The method of claim 32, wherein step (a) further comprises digesting said proteinaceous preparation to produce a complex mixture of peptides.
34. (original) The method of claim 30, wherein said motif of step (b) comprises all or part of a kinase consensus substrate motif or a protein-protein binding motif, or consists of a single phosphorylated amino acid.

APPLICANTS: Rush *et al*
U.S.S.N.: 10/777,893

35. (withdrawn) An immunoaffinity isolation device for the isolation of modified peptides a complex mixture, said device comprising a support comprising at least one modification-specific antibody immobilized to a rigid, non-porous or macroporous resin.
36. (withdrawn) The device of claim 35, wherein said support is selected from the group consisting of a thin capillary column having an internal diameter of about 50 to 300 micrometers and a micropipette tip.
37. (withdrawn) The device of claim 35, wherein said modification-specific antibody comprises a motif-specific, context-independent antibody.
38. (withdrawn) The device of claim 36, wherein said column is adapted to be coupled to an electrospray source on a mass spectrometer.
39. (withdrawn) An antibody that binds ubiquitin fusion degradation protein 1 (UFD1) only when phosphorylated at serine 335, but does not substantially bind to UFD1 when not phosphorylated at this residue.
40. (withdrawn) An antibody that binds protein-tyrosine phosphatase 1c (PTN6) only when phosphorylated at serine 588, but does not substantially bind to PTN6 when not phosphorylated at this residue.
41. (new) The method of claim 1, wherein said modification comprises acetylation, glycosylation, or methylation.